DEVELOPMENT OF THE WALLS IN THE PROEMBRYO OF *PINUS LARICIO*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XCVI

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(WITH PLATES VIII AND IX)

HISTORICAL SKETCH

In 1862 HOFMEISTER (1) gave the principal facts in the development of the proembryo of *Pinus Strobus* and *Pinus Abies*, although he did not touch upon the development of the walls.

STRASBURGER (2) worked out the development of the proembryo in more detail in 1876, and he was the first to recognize the arrangement in tiers. He says that after the four free nuclei have reached the basal end of the egg, and arranged themselves into a plane, the nuclei opposite each other exert an influence upon the surrounding protoplasm, giving it a parallel-striped appearance, and that this influence is also exerted upon the protoplasm of the egg mass above the nuclei; a row of granules then appears upon the parallel stripes; at the metaphase of the first division, after the nuclei have reached the base of the spore, the stripes and row of granules toward the egg-mass disappear, while those between the nuclei persist, form membranes, and become the permanent vertical walls.

In 1898 BLACKMAN (3), in describing the proembryo of *Pinus silvestris*, said that after the four nuclei have passed to the bottom of the egg they become surrounded by protoplasmic fibers which radiate from the nuclei into the cytoplasm; they have no definite arrangement, but are mostly directed away from the base of the egg. At a later stage, when the four nuclei have arranged themselves in one plane, the fibers also are confined to the basal end of the egg and form a mass in which the nuclei lie, the mass projecting a little above the nuclei; after this the two walls are formed at right angles to each other and to the base of the egg, so that each nucleus lies in one end of a shaft which is open toward the main mass of the egg; after the walls have been formed the fibers disappear. He says that he could not

determine exactly what part the fibers play in the formation of these walls, but of their connection with them there can be no doubt.

The next work on Pinus was that of CHAMBERLAIN (**4**), who describes the four nuclei in moving toward the base of the egg as being invested by strong fibers which are directed tangentially. "Upon reaching the base of the oospore the four nuclei arrange themselves in a plane and become ensheathed by fibers derived from the nuclear membrane. Two walls are then formed at right angles to each other and to the base of the spore, each nucleus thus being separated from the others, but freely exposed to the general cytoplasm of the spore." He quotes BLACKMAN (**3**) as thinking that these fibers have some intimate connection with the formation of the walls, and thinks that the appearance of these first incomplete walls is quite apart from nuclear division.

In 1904 Miss FERGUSON (**5**) stated that "after attaining full size, the four nuclei pass to the base of the oosphere." "During their descent many fibers arise in the cytoplasm surrounding the nuclei," and she quotes BLACKMAN as suggesting a relation between these fibers and the walls that arise later, but says that she finds no connection between the two. In regard to the formation of the first walls she says: "During mitosis the deeply staining substance surrounding these nuclei condenses into large, irregular masses at the periphery of the nucleus. When the eight nuclei are formed this deeply staining material collects about them and extends in irregular strands into the cytoplasm. Each nucleus is now surrounded by its own cytoplasm, though no cell-walls have yet been laid down." Further, she says: "In the five species of pines which I have studied, cell-walls do not arise until after eight nuclei have been formed. The deeply staining cytoplasmic substance appears to be repelled from all sides of these nuclei and is deposited in lines which indicate the position of the future cell-walls; the cell-membranes appear to arise by a direct transformation of this substance."

DEVELOPMENT OF WALLS

The material for the following study of the development of walls in the proembryo of *Pinus Laricio* was collected during the summers of 1905 and 1906. It was taken from different trees and localities.

Preparations were also loaned to the writer by Miss LULU PACE, and preparations and material by Professor W. E. PRAEGER.

After the four nuclei have passed to the basal end of the egg, each nucleus is surrounded by a thick sheath of fibers, forming a sphere with the nucleus in the center; these fibers, according to CHAMBERLAIN, are derived from the nuclear membranes. The fibers next to the nuclei disappear first; where the sheaths come in contact, between the nuclei, they form a pseudo-wall, giving the appearance, under low power, of a vertical wall (*fig. 1*). It was this, no doubt, which was seen by the previous observers, STRASBURGER, BLACKMAN, and CHAMBERLAIN, and described by them as a vertical wall.

The cytoplasm surrounding the nuclei at this time is of a uniform granular consistency, with small vacuoles. When the first division of the four nuclei has reached the metaphase, every vestige of these fibers has disappeared (*fig. 2*). The spindles are blunt and broad, showing a tendency towards multipolarity, though considerable variation exists in the form of the spindles during this division. A marked change has taken place in the surrounding protoplasm, which has become charged with very coarse, deeply staining granules. In the early telophase these coarse granules still persist, but there is a larger clear space, filled with fibers, around each spindle (*fig. 3*).

In late telophase (*fig. 4*) the cell plate, from which the cross wall is formed, has been developed. The nuclear membrane has appeared, and above each daughter nucleus is a deeply staining granular area. Fibers radiate from the daughter nuclei in all directions and the coarse granules seem to be strung along the fibers and pushed away by them. The spindle fibers gradually disappear until they are visible only on each side of the cell plate, as is shown in *fig. 5*. The granular area surrounding each nucleus in *fig. 4* is now replaced by an area much more finely granular.

The formation of the vertical walls is begun at this stage. They begin at the cross walls and extend in both directions until they reach the base of the egg on one side, and extend somewhat beyond the nuclei on the other (*fig. 8*). These walls are undoubtedly formed upon secondary fibers, which can be seen coming together in *fig. 4*, and more distinctly in *fig. 5*, although no granules have yet been formed to show the position of the cell plate; these are shown distinctly in

fig. 9. *Figs. 6* and *9* show the fibers radiating from the nuclei to the walls. The vertical walls in each tier are formed by the fibers from the nuclei of their respective tiers, as is shown in *fig. 8*. As a rule the cross wall is formed somewhat in advance of the vertical wall (*figs. 4–9*). *Fig. 8* shows the cross walls completed, except at the edges, while the vertical walls are still in an early stage of development; this is also shown in *fig. 9*. The cross walls are formed in the usual way, each on its own spindle, as is shown in *fig. 6*. These walls, therefore, are formed in connection with the first division of the four nuclei. It might be said that the proembryo passes from the four-nucleate stage to the eight-celled stage were it not for the fact that the four nuclei farthest from the base of the egg are still freely exposed to the egg mass.

The very coarse granules gradually disappear, as shown in *figs. 4–9*, while the deeply staining areas first seen in *fig. 4* increase in size until in *figs. 8* and *9* they entirely surround the nuclei and extend irregularly into the surrounding cytoplasm.

The second division in the base of the egg, as a rule, takes place in the exposed tier (*fig. 10*), although it may take place in the basal tier, as shown in *fig. 11*. The cross and vertical walls are formed as in the previous division.

The next and last division occurs in the basal tier (*fig. 12*), and, so far as has been reported, it never takes place in either of the other two tiers. This sequence of divisions is also reported by HOFMEISTER (**1**) in *Pinus Strobus* and by MIYAKE (**6**) in *Picea excelsa*. The last division in the proembryo of *Pinus Laricio* is not always simultaneous in the four nuclei, as has heretofore been reported; one section showed two nuclei in metaphase and the other two in late telophase.

The proembryo has now completed its development and consists of three completely walled tiers of four cells each, and the tier of four nuclei separated from each other by vertical walls but free toward the egg mass.

SUMMARY

1. The cross walls in the proembryo of *Pinus Laricio* are formed in the usual way, on cell plates formed on the spindles in the division of the nuclei, and the first cross walls come in a little in advance of the vertical walls.

2. The vertical walls in each tier are formed by the secondary fibers from the nuclei of their respective tiers.

3. The first cross walls and the first vertical walls are formed in connection with the division of the first four free nuclei at the base of the egg.

4. The second division in the basal end of the egg may take place in either tier.

5. In the last division the four nuclei do not always divide simultaneously.

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EXPLANATION OF PLATES VIII AND IX

PLATE VIII

FIG. 1. Section showing fibers between nuclei that give appearance of vertical wall. ×225.

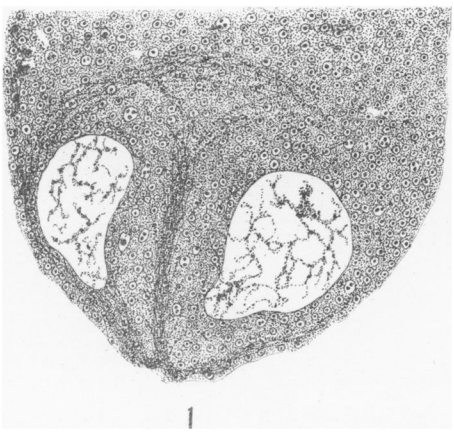
FIG. 2. Metaphase of first division, showing entire disappearance of fibers, blunt broad spindles, and coarse granular substance. ×610.

FIG. 3. Telophase of same division; fibers radiating in all directions; cell plate not yet visible. ×355.

FIG. 4. Appearance of cell plate; nuclear membranes; dark areas above daughter nuclei; fibers radiating from nuclei and coarse granules strung along fibers; secondary fibers coming together in the center, upon which is formed the vertical wall. ×390.

FIG. 5. Later stage in cross walls and beginning of vertical walls. ×365.

FIG. 6. Later stage in formation of vertical walls; fibers radiating from nuclei. ×390.



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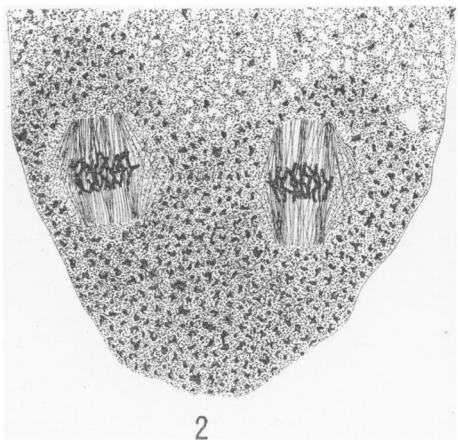

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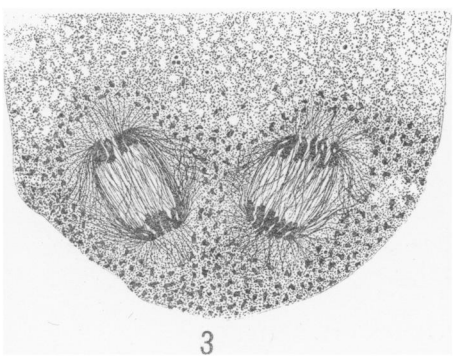

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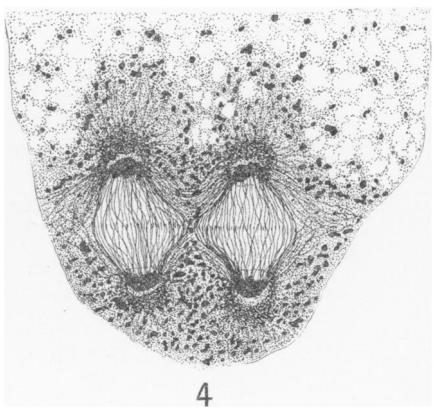

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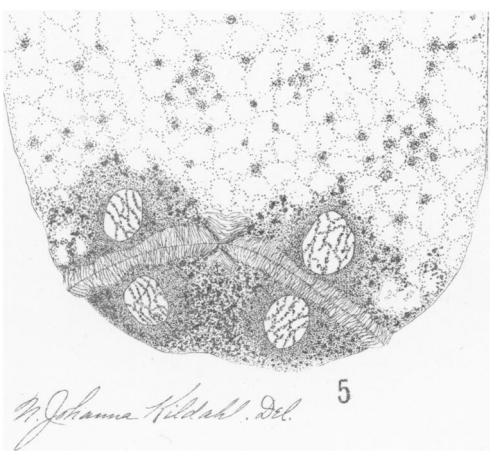

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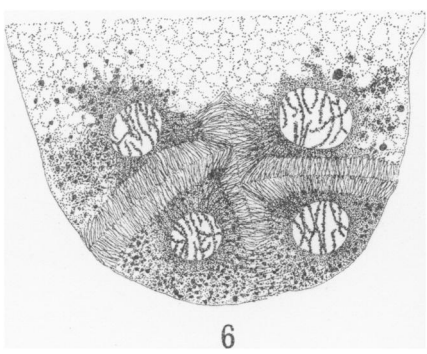

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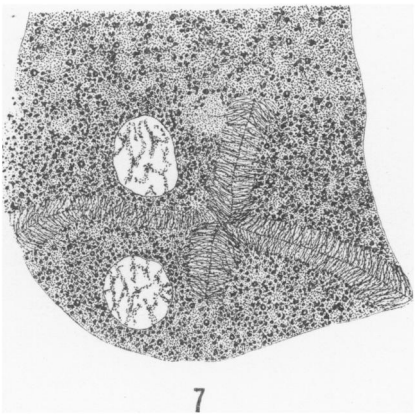

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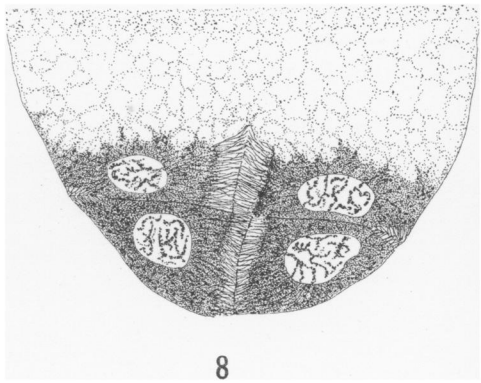

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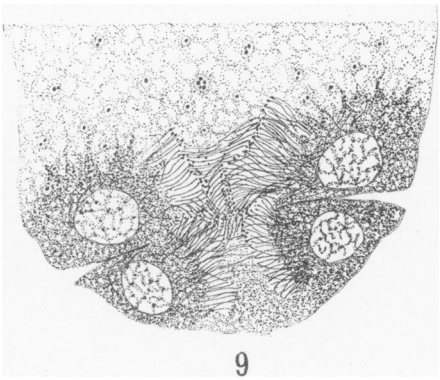

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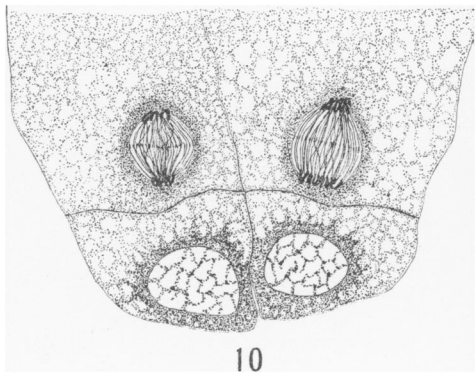

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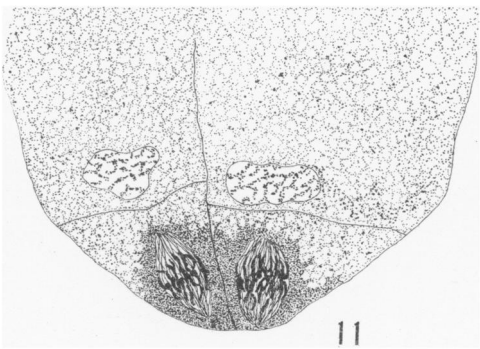

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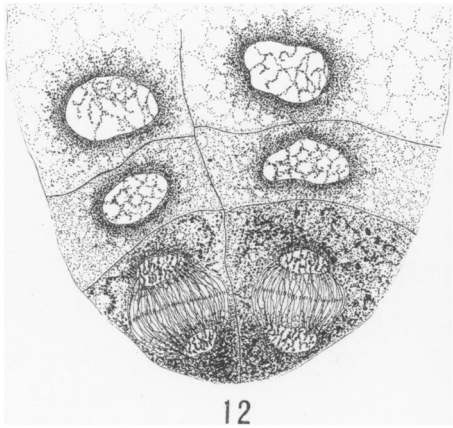

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PLATE IX

FIG. 7. Section showing the two walls more nearly in the same stage, but the cross wall a little in advance of the vertical. ×425.

FIG. 8. Section showing the vertical walls of each tier formed by the fibers from the nuclei of their respective tiers; the cross wall nearly complete. ×350.

FIG. 9. Same as *fig. 8;* the broad effect of the vertical wall caused by the section being cut a little to one side of the crossing of the two vertical walls. ×350.

FIG. 10. Second division. ×425.

FIG. 11. Second division. ×355.

FIG. 12. Third division. ×393.